PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: SKOULTCHI

Serial No.: 08/102,390 Group Art Unit: 1804

Filed: August 5, 1993 Examiner: Ziska, S.

For: PRODUCTION OF PROTEINS USING HOMOLOGOUS RECOMBINATION

Atty Docket No.: 7639-017/Cell 3.2

DECLARATION OF DANIEL J. CAPON

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Daniel J. Capon, declare that:

1. I am Senior Vice-President and Chief Technical Officer at Cell Genesys, Inc. ("Cell Genesys"). I make this declaration in support of Applicant's Petition To Make Special Pursuant To 37 C.F.R. §1.102(d), filed concurrently herewith.

2. I was awarded the degree of Doctor of Philosophy in Biochemistry from the Massachusetts Institute of Technology in 1981. From 1981 to 1990, I worked at Genentech, Inc., in molecular biology research, achieving the position of staff scientist, the most senior scientific position at that company. From 1990 to 1994, I was Vice-President of Research at Cell Genesys, and since 1994 I have been Senior Vice-President and Chief Technical Officer at Cell Genesys, where I am responsible for research and development strategy.

3. I am familiar with the various methods for inserting DNA sequences into the genome of mammalian host cells, including homologous recombination. I am also familiar

with recombinant DNA methods that are currently being utilized in the field of gene therapy.

4. I have read and am familiar with that portion (page 1, lines 10-21) of PCT International Publication WO 94/12650, entitled "Activating expression of an amplifying endogenous gene by homologous recombination" (Applicant: Transkaryotic Therapies, Inc. ("TKT")) [pages 1-6 attached hereto as Exhibit A] that states:

> Presently available approaches to gene therapy make use of infectious agents, such as retroviral vectors, which include the genetic material to be expressed. Such approaches have limitations, such as the potential of generating replication-competent virus during vector production; recombination between the therapeutic virus and endogenous retroviral genomes, potentially generating infectious agents with novel cell specificities, host ranges, or increased virulence and cytotoxicity; independent integration into large numbers of cells, [and] increasing the risk of a tumorigenic insertional event[.]

5. I have also read and am familiar with the claims as amended in an Amendment Under 37 C.F.R. §1.115, filed on June 1, 1994, in the above-identified application.

6. The invention disclosed in the above-identified application relates to the safety of research in the field of recombinant DNA by providing methods and compositions for transferring different nucleotide regulatory sequences into mammalian host cells to control endogenous gene expression without requiring the use of viral or retroviral vectors.

7. The invention disclosed in the above-identified application further relates to the safety of research in the field of recombinant DNA by providing methods and compositions

for transferring different nucleotide regulatory sequences into mammalian host cells in a precisely targeted manner using homologous recombination, thus reducing or eliminating the possiblity of multiple integrations at random sites in the mammalian host cell genome.

8. I have also read and am familiar with:

(a) TKT's S-1 Registration Statement, as filed with the Securities and Exchange Commission on July 26, 1993 [pertinent pages attached hereto as Exhibit B] that describes TKT's gene activation technology;

(b) a published report entitled "TKT Scientists Deliver Erythropoietin (EPO) By Gene Therapy; Novel Gene Activation Technology Eliminates Need For EPO License" which appeared in PR Newswire's "Today's Headlines" on January 12, 1994 [Exhibit C]; and

(c) a published report entitled "Mice Produce EPO In Gene Therapy Study" which appeared in BioWorld Today, Vol.5, No. 9, pp. 1-2, on January 13, 1994 [Exhibit D].

9. On or about January 12, 1994, Cell Genesys first became aware that TKT was performing Applicant's method for producing endogenous proteins using homologous recombination in mammalian host cells.

10. TKT's gene activation technology is described in Exhibits B-D:

(a) Exhibit B, page 4, second paragraph, states:

> TKT has developed proprietary gene targeting and gene isolation technologies to enhance its gene therapy products. Gene targeting is a technique in which genes are inserted or replaced at a chosen site on a given chromosome (emphasis added).

From this statement, TKT appears to have used its gene activation technology to produce and express "fusion genes", *i.e.*, nucleotide sequences in which different regulatory elements are combined with endogenous genes encoding proteins such as human EPO, human Factor VIII, *etc.*, in mammalian host cells, in this case, human fibroblast cells.

(d) Exhibit C, fourth paragraph, states:

> TKT's proprietary gene activation technology involves the surgically precise modification of a patient's cells. A piece of DNA is inserted adjacent to the natural EPO gene in normal cells -- this piece of DNA represents a genetic 'switch' that turns on EPO production in the cells. The technology is an extension of the work on the genetic modification of normal human cells developed at TKT over the past five years. The technology can be applied to the activation of essentially any human gene, the company noted (emphasis added).

(e) Exhibit D, page 2, first full paragraph, states:

> [TKT's President and Chief Executive Officer, Michael Forrest], explained that within the skin fibroblast cell there is a control region - a gene sequence - that tells the cell not to make EPO since EPO is not naturally produced in skin fibroblast cells. He said TKT is able to insert its own control region in the cell that deletes the existing control region. The new control region instructs the cell to produce EPO (emphasis added).

From these two statements, TKT's gene activation technology appears to involve the replacement of a wild-type (endogenous) nucleotide regulatory element, *i.e.*, the "genetic switch" or "control region" described above, that is normally associated with an endogenous gene, with a different nucleotide regulatory element that activates gene expression. The

insertion of the different nucleotide regulatory element "adjacent" to the natural gene indicates that this regulatory element is inserted so that it is operatively associated with the endogenous gene of the mammalian host cell. That "this piece of DNA ... turns on EPO [protein] production in the cells" indicates that expression of the endogenous gene is controlled by the inserted different regulatory element.

(f) Exhibit B, page 21, sixth paragraph, states:

> Following transfection, a single cell selected from a pool of transfected cells is cloned (i.e., copied) and propagated, resulting in a uniform population of identical cells for implantation. ... [T]he Company clones a cell that is capable of producing the therapeutic protein at desired levels (emphasis added).

Thus, following transfection, mammalian host cells are selected in which there has been successful integration of the different nucleotide regulatory sequence so that it is operatively associated with, and controls expression of, the endogenous gene.

(g) Exhibit D, page 1, first paragraph, states:

> Mice that received implants of skin cells modified to contain the erythropoietin gene have produced therapeutic levels of EPO for one year, Transkaryotic Therapies Inc. reported Wednesday ... (emphasis added).

(h) Exhibit C, fifth paragraph, states:

> TKT is currently engaged in pre-clinical studies of the company's EPO gene activation product for the treatment of severe anemia and is discussing additional gene activation targets with corporate partners (emphasis added).

From these two statements, it appears that TKT is presently using its gene activation technology to produce proteins in mammalian host cells.

11. In view of the pertinent portions of the description of TKT's gene activation technology, as recited and described hereinabove in paragraph 10(a)-(h), I believe that TKT's gene activation technology produces a mammalian host cell, and is a method for producing proteins in a mammalian host cell, in which expression of an endogenous gene in the host cell is altered, *i.e.*, activated, by replacing a wild-type nucleotide regulatory element in the host cell with a different nucleotide regulatory element via homologous recombination. I further believe that the different regulatory element is integrated into the mammalian host cell genome so as to be operatively associated with the endogenous gene of the mammalian host cell, that expression of the endogenous gene is controlled by the integrated different regulatory element, and that altered mammalian host cells with the correctly integrated different nucleotide regulatory element are selected and propagated.

12. The undersigned declares further that all statements made herein of his own knowledge are true, that all statements made on information and belief are believed to be true, and further that these statements and the like so made

are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such wilful false statements may jeopardize the validity of this application or any patent issuing thereon.

Date: 12/28/94

Dennis J. Capone

Attachments:

Exhibit A: PCT Publication WO 94/12650, entitled "Activating expression of an amplifying endogenous gene by homologous recombination" to Transkaryotic Therapies, Inc. (pp. 1-6).

Exhibit B: S-1 Registration Statement for Transkaryotic Therapies, Inc., as filed with the Securities and Exchange Commission on July 26, 1993 (pp. 1-4, 21-23).

Exhibit C: Report entitled "TKT Scientists Deliver Erythropoietin (EPO) By Gene Therapy; Novel Gene Activation Technology Eliminates Need For EPO License." PR Newswire's "Today's Headlines," published January 12, 1994.

Exhibit D: Report entitled "Mice Produce EPO In Gene Therapy Study." BioWorld Today, Vol.5, No. 9, pp. 1-2, published January 13, 1994.